Chemistry of Natural Compounds, Vol. 33, No. 1, 1997

^1H AND ^{13}C SPECTROSCOPY IN THE STUDY OF FLAVAN-3-OLS, PROANTHOCYANIDINS, AND THEIR DERIVATIVES

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UDC 543.42.25+541.63+541.67

This review generalizes information on the use of the ^1H and ^{13}C NMR methods for studying the structure, stereochemistry, and conformation of flavan-3-ols, proanthocyanidins, and their derivatives.

Among spectral methods playing an important role in the identification of natural compounds with various degrees of complexity, proton magnetic resonance (PMR) spectroscopy occupies a leading place with respect to its informativeness. This circumstance is particularly important in the investigation of the structures, configurations, and conformations of natural compounds in solutions, where their biological action is usually manifested. At the present time, thanks to the development of theoretical aspects and the introduction into practice of new pulse procedures, the possibilities of magnetic resonance spectroscopy have expanded still further.

Chemical and spectral methods of identification of a fairly widespread class of natural flavonoids have been described in reviews by Markham [1, 2], Mabry [3], Harborne [112], Pelter [4], and Agrawal and Rastogi [5]. The theoretical principles of NMR spectroscopy have been expounded in a number of monographs and reviews [6-20].

PMR spectroscopy has indisputable advantages: a high natural content of protons, ^1H, with spin 1/2 — 99.98%; the fairly large magnetogyric ratio of $2.675 \cdot 10^8$ rad/T·s; and a relatively high sensitivity as compared with other nuclei. The main parameters of proton magnetic resonance spectra are the chemical shift (CS) and spin-spin coupling constant (SSCC). Their generally known link with the structures of organic molecules is used for the study of structural features in solutions.

In a molecule, different nuclei of one type — for example, protons — are screened differently according to their electronic environments, the presence of various functional groups, the type of hybridization, the presence or absence of ring currents, etc. Each of these factors makes its own contribution to the screening constant of the nucleus and, in sum, they determine its chemical shift. Practically every structural fragment has a region of chemical shifts characteristic for it. And in spite of the fact that these regions may overlap, with spectrometers having a high working frequency, and also by using special procedures, it is possible to separate the signals of the majority of proton groups.

The study of SSCCs in PMR spectra permits the relative configurations of substituents in rings to be found, the conformations of natural and synthetic compounds to be determined, and their structures to be established.

Recently, for the assignment of signals ever wider use has been made of paramagnetic shift reagents: titanium chloride and compounds of europium and other lanthanoids, which are capable of inducing considerable changes in CSs — of the order of several tens of parts per million. When they are used, account must be taken of a possible broadening of the signals, since the majority of shift reagents are paramagnetic, and also of the possibility of their spatial descreening influence on various atoms.

The use of PMR spectroscopy continues to remain extremely important in the investigation of the structures, configurations, and conformations of practically all classes of organic compounds.

PMR Spectroscopy of Flavan-3-ols

The polymeric proanthocyanidins and their low-molecular-mass components, flavan-3-ols, are an important class of natural compounds. They are widely distributed in nature, being secondary metabolites of higher plants [21-23]. By passing

Translated from Khimiya Prirodnykh Soedinenii, Vol. 33, No. 1, pp. 16-33, January-February, 1997. Original article submitted May 1, 1995.

0009-3130/97/3301-0011$18.00

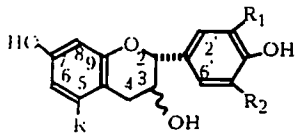
TABLE 1. CSs (δ, ppm) and SSCCs (J, Hz) of the Protons in the PMR Spectra of (+)-Catechin (**1**), (−)-Epicatechin (**3**), (−)-Epigallocatechin (**4**), and (+)-Afzelechin (**6**)

H	2	3	4	6	8	2′	5′	6′	Literature
1 (C2)	4.57 d $J_{2,3}$=8	4.02 m	α2.96 dd $J_{4,3}$=6 $J_{4,4'}$=16 β2.50 dd $J_{4,3}$=8	5.90 d $J_{6,8}$=2	6.04 d $J_{8,6}$=2	6.90 d $J_{2',6'}$=2	6.80 d $J_{5',6'}$=8	6.72 dd $J_{6',5'}$=8 $J_{6',2'}$=2	54*
6 (C2)	5.02 $J_{2,3}$=9.0	4.00 $J_{3,4}$=5.05	3.09 $J_{4,4'}$=16.0						55***
3	4.88 s	4.22 m	2.80 m	6.12 d $J_{6,8}$=2	6.22 d $J_{8,6}$=2	7.04 d $J_{2',6'}$=2	6.86 d $J_{5',6'}$=8	6.78 dd $J_{6',5'}$=2 $J_{6',2'}$=8	49** 52
4	4.81 s	4.18 m	2.80 m	5.91 m $J_{6,8}$=2	6.02 d $J_{8,6}$=2	6.57 s	–	6.57 s	51**

*Solvent a mixture of acetone-d_6 and D_2O; here and in Tables 2—6 the conformations of rings *C* following from SSCC values are shown in parentheses.

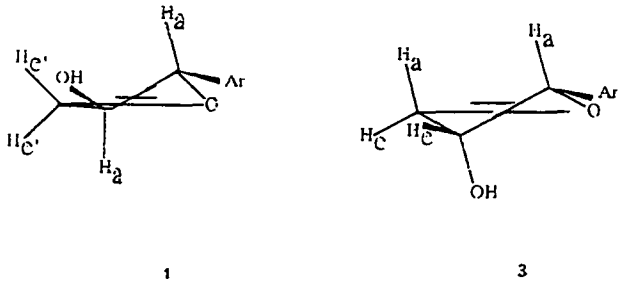
**Solvent acetone-d_6.

***Solvent $CDCl_3+CCl_4$.



3- OH	R	R_1	R_2
1. β	OH	OH	H
2. β	OH	OH	OH
3. α	OH	OH	H
4. α	OH	OH	OH
5. α or β	H	OH	H
6. α or β	OH	H	H
6a. α or β	H	OH	OH

Fig. 1. Structures of the flavan-3-ols (**1-6a**).



1 **3**

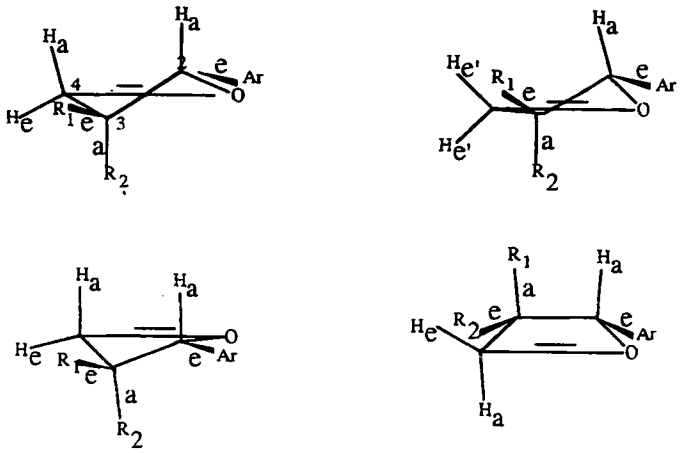
Fig. 2. Conformations of ring *C* and orientations of the substituents in (**1**) and (**3**).

into anthocyanidins and flavanoids, these compounds, together with other pigments, are responsible for the colors of flowers and fruits [24], and they are present in practically all organs of trees, bushes, and herbs.

The investigation of proanthocyanidins is of great practical importance, since they possess a broad spectrum of biological properties. They have long been used as tanning agents [21, 25-27]. At the present time, proanthocyanidins are used as astringents [28] and as antihypoxic drugs [29] with an anticholinergic action. The conformational mobility of the proanthocyanidins increases the possibility of their intermolecular interaction with proteins [30]. They are capable of modifying the activity of enzymes by blocking individual sections of their surface [31]. Proanthocyanidins are used in practice for the quantitative precipitation of protein [32], including precipitation for the quantitative determination of protein in milk [33]. There are reports in the literature that in biological materials proanthocyanidins are capable of binding the proteins of tumor cells to

TABLE 2. Vicinal SSCCs (Hz) of the Protons of Ring *C* of Flavan-3-ols in Various Conformations

Conformation	$J_{2,3}$	$J_{3,4\alpha}$	$J_{3,4\beta}$
(+)-Catechin			
C3-pentacoplanar (C3)	10.0	2.5	10.0
half-chair (HC)	10.0	3.2	10.0
C2-pentacoplanar (C2)	10.0	6.0	7.8
half-boat (HB)	6.0	5.1	<1
(−)-Epicatechin			
C3-pentacoplanar (C3)	2.2	<1	2.2
half-chair (HC)	<1	3.2	3.0
C2-pentacoplanar (C2)	<1	<1	5.6
half-boat (HB)	7.7	9.9	3.9



1. R_1 =OH; R_2 =H
4. R_1 =H; R_2 =OH

Fig. 3. Conformations of ring *C* in (+)-catechin and (−)-epicatechin.

a greater degree than those of normal cells [34]. They exhibit the properties of antibiotics [34] and possess antiviral activity [35-37]. Complex compounds of proanthocyanidins with metals are used for the introduction of trace elements into the soil [38]. On the other hand, polyphenols are alimentary detergents and may lower the activity of digestive enzymes [39-41], exhibiting a reductase activity in relation to them [42].

One of the main low-molecular-mass fragments of the proanthocyanidins is (+)-catechin, which forms hydrogen bonds with protein molecules by means of the *ortho*-diphenyl hydroxy groups C-3′-OH and C-4′-OH and is capable of stabilizing the β-pleated structure of a protein [43]. It may also act as an anticoagulant [44]. Catechin, together with other flavanoids, belongs to the group of vitamins P [45]. In addition, it is capable of immunomodulating T-lymphocytes [46].

All the biochemical properties of phenols and polyphenols that have been mentioned are promoting interest in the further investigation of features of their structure in such countries as the USA, Japan, Australia, the SAR, and others. In this connection, the search for an interrelationship between the structure of this class of compounds and their spectral properties is an urgent one.

Chemical Shifts of the Protons of (+)-Catechin, (−)-Epicatechin, and (−)-Epigallocatechin

The main structural blocks of the proanthocyanidins are (+)-catechin (**1**), (+)-gallocatechin (**2**), (−)-epicatechin (**3**), and (−)-epigallocatechin (**4**) (Fig. 1). Mirror isomers differ by opposite signs of the rotation of the plane of polarized light. Epimers are stereoisomers in which one asymmetric center is inverted, i.e., diastereoisomers.

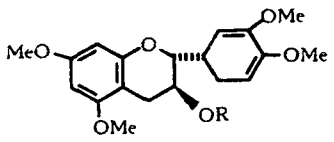
TABLE 3. CSs (δ, ppm) and SSCCs (J, Hz) of the Protons in the PMR Spectra of Flavan-3-ol Ethers and Esters

H	2	3	4	6	8	2′	5′	6′	Literature
7 (C2)	4.60d $J_{2,3}$=8	3.95 m	α3.00dd $J_{4,3}$=6 $J_{4,4'}$=16 β2.48dd $J_{4,3}$=8	6.09d $J_{6,8}$=2	6.13d $J_{8,6}$=2	6.91	6.91	6.85	48*
8 (HC)	4.91 $J_{2,3}$=1	4.20 $J_{3,4}$=3.4	2.86						69**
8a	4.91 s $J_{2,3}$=1	4.24 m	3.00 m	6.12d $J_{8,6}$=2	6.17d $J_{8,6}$=2				74*
9 (HC)	5.70 $J_{2,3}$=6.7	5.35 $J_{3,4a}$=7.0	α2.90 e $J_{4,4}$=16.8 β 2.67a $J_{3,4e}$=3.4						73***
10 (HC)	4.99 $J_{2,3}$=1.3	5.43 $J_{3,4}$=3.3	2.91						69**
11 (HC)	4.97 $J_{2,3}$=0.2	5.44 $J_{3,4}$=3.4	2.94						69**
12	5.06 s	5.40 m	2.8–3.0 m	6.56′ $J_{8,6}$=2	6.66′ $J_{8,6}$=2	7.20		7.20	75*
13 (C2)	5.13d $J_{2,3}$=8	5.44m $J_{3,4e}$=6 $J_{3,4a}$=9	αe2.90dd βa2.75dd $J_{4,4}$=16	6.08d $J_{8,6}$=3	6.17d $J_{8,6}$=3				76
14	5.14 s	5.56 m	2.98 m	6.04d $J_{8,6}$=3	6.08d $J_{8,6}$=3	7.06 $J_{2',6'}$=3	6.76	6.90 $J_{5',6'}$=8	69**

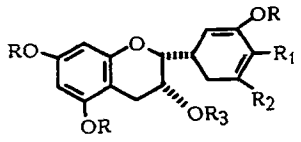
*Solvent $CDCl_3$; for (12) D_2O+acetone-d_6.

**Solvent CCl_4.

***Solvent $CDCl_3$, temperature 100 °C. The signals may be interchanged. The underlined figures denote esterification of the hydroxy group at C-3.



7. R=H
9. R=Ac



	R	R_1	R_2	R_3
8.	Me	OMe	H	H
8a.	Me	H	H	H
10.	Me	OMe	H	Ac
11.	Me	OMe	OMe	Ac
12.	Ac	OAc	OAc	Ac

Fig. 4. Structures of the flavan-3-ol ethers and esters.(8-12).

Sometimes encountered in plant materials are fisetinidol — 3,3′,4′,7-tetrahydroxyflavan (5) —, afzelechin — 3,4′,5,7-tetrahydroxyflavan (6) —, and robetiniddol — 3,3′,4′,5′,7-pentahydroxyflavan (6a) —, which may also form components of proanthocyanidins.

A comparative analysis of the chemical shifts of the protons of catechins (see Table 1) shows that on the inversion of the C-3-OH group — i.e., on passing from (+)-catechin (1) [44, 48, 49, 51, 54, 68] to (−)-epicatechin (3) [47-52, 110, 111] the induced chemical shift (ICS) of H-3 is +0.2 ppm [48, 51]. (Here and below, unless specially stated in the text, the ICSs were calculated by us from results given in the tables or in the text.) The positive sign of the ICS of the H-3 proton is due mainly to the change in its orientation from axial to equatorial (see Fig. 2). Some contribution to the paramagnetic shift of H-2

TABLE 4. CSs (δ, ppm) of the H-6 and H-8 Protons for C-8 and C-6 Derivatives, Respectively, of (+)-Catechin*

C-3-OR	Substituent	CS of H-6	CS of H-8	$\Delta\delta=\delta_{H\text{-}8}-\delta_{H\text{-}6}$
H	C-β-Glucose	6.08	5.96	–0.12
H	Br	6.21	6.40	0.19
Ac	Br	6.20	6.47	0.27
		6.19	6.45	0.26
	**	6.68	6.77	0.19
CH_2-Ph	Br	6.22	6.41	0.19
H	OH	6.22	6.32	0.10
CH_2-Ph	OH	6.18	6.32	0.14
H	O-Ac	6.18	6.36	0.18
Ac	O-Ac	6.19	6.40	0.21
CH_2-Ph	O-Ac	6.15	6.34	0.19
H	CO_2Me	6.10	6.32	0.22
Ac	CO_2Me	6.11	6.32	0.21
CH_2-Ph	CO_2Me	6.10	6.32	0.22
Ac	Br (t 149°C)	6.69	6.75	0.06
Ac	Br***(t 149°C)	6.17	6.41	0.24
Gambiriin		6.08	6.02	–0.06
H	i****	6.25	6.12	–0.13
H	Isoprenyl****	6.10	6.00	–0.10

*If not specially stated, all the aromatic hydroxylic functions were methylated.

**The aromatic phenolic groups were acetylated.

***Only the 4′-OH group was methylated (t 149°C).

****All the hydroxylic functions were acetylated.

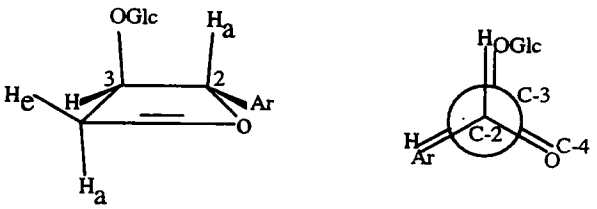


Fig. 5. Conformation of (+)-catechin 3-O-glucoside.

by +0.3 ppm is connected with the change in the orientation of the OH group at C-3. Similar values of the ICSs are observed with a change in the orientation of the hydroxy group in 2-arylcyclohexan-1-ols [53].

An interesting effect is shown on passing from (+)-catechin to afzelechin: we observe a paramagnetic shift of the H-2 proton in ring *C* by approximately 0.45 ppm. This is apparently due to the absence of an OH group in the *meta*- position of ring *B*.

On considering the chemical shifts of the aromatic protons of compounds (**1**), (**3**), and (**4**), we observe a paramagnetic shift of the H-2′ proton by +0.14 ppm for (−)-epicatechin as compared with (+)-catechin. This is possibly connected with the spatial influence of the C-3-OH group, which may be close to this proton in (−)-epicatechin. In (+)-catechin and (−)-epicatechin the pattern of chemical shifts and the spin splitting of the protons of ring *B* are typical for flavonoid 3′,4′-diols.

Analysis of the spectral characteristics of the aromatic protons also showed that the signal of the H-8 proton in aromatic ring *A* is shifted downfield in comparison with the H-6 signal by approximately 0.10-0.15 ppm. This phenomenon can be explained by the large negative inductive effect of the cyclic ether bond at C-9 in comparison with the analogous effect of the hydroxy group at C-5.

The chemical shifts of the protons of rings *A* and *C* of (−)-epigallocatechin are close to the corresponding CSs of the protons of (−)-epicatechin. The H-2′ and H-6′ protons of the symmetric gallic system of ring *B* give the common signal at 6.75 ppm that is characteristic for the gallic type of oxidation of ring *B*.

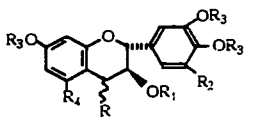
Thus, by a comparative analysis we have elucidated the influence of structural factors of the flavan-3-ols on the proton magnetic resonance chemical shifts and have revealed their main features.

TABLE 5. CSs (δ, ppm) and SSCCs (J, Hz) of the Protons in the PMR Spectra of Flavan-3-ols with the 2,3-*trans*- Configuration having a Substituent in Position 4

H	2	3	4	6	8	2′	5′	6′	Litera-ture
15 (HC) ICS	4.62 d $J_{2,3}$=9.0 0	4.28 t $J_{3,4}$=9.0 +0.3	β4.68 d $J_{4,3}$=9.0 +2.2	6.02 d	6.20 d	6.69 s	–	6.69 s	75*
15a (HC) ICS	5.00 d $J_{2,3}$=10 0	5.78 t $J_{3,4}$=9.6 +0.3	β4.70 d $J_{4,3}$=9.4 +2.2	6.62 d	6.79 d	7.33 s	–	7.33 s	75*
16 (C2) ICS	4.96 d $J_{2,3}$=9.7 –0.8	–	α4.94 d $J_{4,3}$=5.8 +2.1						89*
16a (C2) ICS	4.94 d $J_{2,3}$=10 –0.8	5.45 dd ΣJ=16.3 0	α5.04 d $J_{4,3}$=6.3 +2.1						90*
16a (C2) ICS	5.13 d $J_{2,3}$=8.0 –0.7	5.53 dd ΣJ=13.0 0	α4.82 d $J_{4,3}$=5.0 +1.9						90*
17 (C2) ICS	4.49 d $J_{2,3}$=8 –0.1	4.15 m +0.1	β4.00 d $J_{4,3}$=7 +1.5	5.96 d $J_{8,6}$=2	6.07 d $J_{8,6}$=2	6.90 $J_{2',6'}$=3	6.82 $J_{5',6'}$=8	6.73	91
18 (C2)	4.94 d $J_{2,3}$=10 +0.4	4.1 m +0.1	α4.38 d $J_{4,3}$=4 +1.9	5.89 d $J_{6,8}$=2	6.03 d $J_{8,6}$=2	6.93 $J_{2',6'}$=3	6.84 $J_{5',6'}$=8	6.76	91
19 (C2) ICS	4.62 d $J_{2,3}$=9.9 0	3.88 m –0.1	β5.00 d $J_{4,3}$=7.9 +2.5	5.97 d J=2.3	5.83 d J=2.3				89
20 (HC) ICS	4.96 d $J_{2,3}$=10.4 +0.5	4.10 m +0.15	α4.68 d $J_{4,3}$=3.5 +1.7	6.10	6.10				89*

*Solvent $CDCl_3$; the phenolic groups were methylated; the underlined figures indicate that the hydroxy group at C-3 was esterified.

**Solvent $CDCl_3$, t 110°C.



R	R_1	R_2	R_3	R_4
15. α Phl	H	OMe	Me	OMe
15a. α Phl	Ac	OAc	Ac	OAc
16. β Phl	H	H	Me	OMe
16a. β Phl	Ac	H	Me	H

R	R_1	R_2	R_3	R_4
17. α S-CH_2-Ph	H	OH	H	OH
18. β S-CH_2-Ph	Ac	OH	H	OH
19. α OH	H	H	H	OH
20. β OH	Ac	H	Me	OMe

Fig. 6. Structures of the flavan-3-ol derivatives (**15-20**).

Conformations of Ring *C* of the Flavan-3-ols and Their Influence on the Spin-Spin Coupling Constants of the H-2, H-3, and H-4 Protons

The stereochemistry of the substituents in ring *C* and the conformation of this ring in flavan-3-ols and proanthocyanidins can be determined by using the SSCCs of the H-2, H-3, and H-4 protons. All possible canonic conformations of the heterocycle of the flavan-3-ols and orientations of substituents in them are given in Fig. 3.

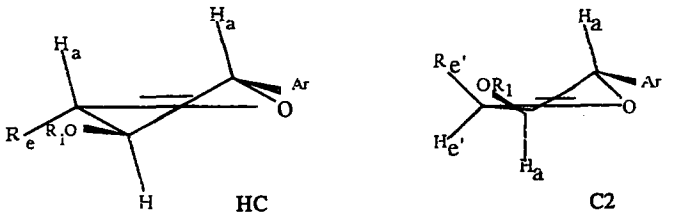


Fig. 7. Conformations of the 4α- and 4β-phloroglucinol derivatives of (+)-catechin.

Drewes et al. [56], and then Foo and Porter [108], using the Karplus equation, calculated the SSCCs of the H-2, H-3, and H-4 protons for various conformations of this ring. Subsequently, other authors also occupied themselves with investigations of the conformations of flavan-3-ols [57-64]. However, we have found no information in the literature on SSCCs for C-3 of the pentacoplanar conformation of (−)-epicatechin, and we have calculated them, making use of the same means. The SSCCs (Hz) of the protons characteristic for various conformations of the flavan-3-ols are given in Table 2 [56, 108].

Theoretically, the most stable conformation for a six-membered ring containing a double bond or conjugated with an aromatic system is a half-chair [65-67]. This is connected with the mutual repulsion of the bonds of the ring and a smaller angular strain. The repulsion of the exocyclic bonds of substituents from the bonds of the ring also explains the greater stability of conformations in which the substituent with the greatest effective volume has an equatorial orientation. Such an orientation ensures its maximum distance from the ring bonds located after one intermediate bond, as can well be seen from Newman projections. However, either conformation can be stabilized by a hydrogen bond, mutual repulsion of the substituents in the ring, or other steric factors, and therefore each concrete case of conformational isomerism requires individual consideration.

The analysis of literature results for the SSCCs of flavan-3-ols [44, 48, 51, 54, 68] that we have given shows that, for example, (+)-catechin has a conformation close to C2-pentacoplanar. This can be explained only by some mutual repulsion of the substituents at C-2 and C-3. As can be seen from Table 1, in the spectra of (−)-epicatechin the signal of the H-2 proton appears in the form of a singlet. This can be observed only in those cases where ring *C* is present in a half-chair or pentacoplanar conformation. In our view, the half-chair is more probable, since in this case it corresponds to all the requirements ensuring the lowest energy of the molecule. There is information on SSCCs in the literature [69] that confirms the existence of just such a conformation.

Thus, the use of vicinal SSCCs of the protons of ring *C* in flavan-3-ols enables us to evaluate not only the orientation of substituents in this ring but also its preferential conformation.

Parameters of the PMR Spectra of Derivatives of Flavan-3-ols with the 2,3-*trans*- Configuration and the Influence of Various Structural Factors on Them

Characteristics of the PMR Spectra of (+)-Catechin Ethers and Esters

Frequently, in the investigation of proanthocyanidins, it is not the PMR spectra of the phenols themselves that are studied but those of their peracetates or of the acetates of their methyl ethers, i.e., when the C-3-OH group is acetylated and the phenolic hydroxy groups are methylated. Then, sometimes, the spectra are obtained under conditions of an elevated temperature or of improved resolution. Table 3 gives the parameters of the PMR spectra of (+)-catechin methyl ether (**7**) and (−)-epicatechin methyl ether (**8**) (only the phenolic groups were methylated) and of 3α-hydroxy-3′,5,7-trimethoxyflavan (**8a**), the acetates of the methyl ethers of (+)-catechin (**9**), (−)-epicatechin (**10**), and (−)-epigallocatechin (**11**), and also the peracetate of (−)-epigallocatechin (**12**).

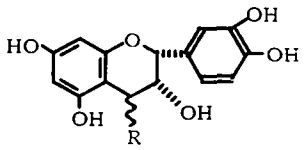
An analysis of the literature on the PMR spectroscopy of methyl and acetyl derivatives of flavan-3-ols [48, 52, 68-72] shows that the C2-pentacoplanar conformation of ring *C* is retained when the phenolic hydroxy groups of (+)-catechin are methylated. Methylation of only the C-4′-OH group induces paramagnetic shifts of the H-5′ and H-6′ protons (+0.15 and +0.1 ppm, respectively). Methylation of all the phenolic groups [48, 54] induces appreciable paramagnetic shifts of the signals of the aromatic protons: H-6 (+0.2 ppm), H-8 (0.1 ppm), H-5′ (+0.1 ppm), and H-6′ (+0.15 ppm).

TABLE 6. CSs (δ, ppm) and SSCCs (J, Hz) of the Protons in the PMR Spectra of Flavan-3-ols with the 2,3-*cis*- Configuration Having a Substituent in Position 4

H	2	3	4	6	8	2′	5′	6′	Literature
21 (C2)	4.93a $J_{2,3}$=1.0	4.22e $J_{3,4}$=4.8	β5.18a $J_{4,3}$=4.8						92*
ICS	0	0	+2.4						
21a (C2)	5.15a $J_{2,3}$=1.6	5.62e $J_{3,4}$=5.4	β6.50a $J_{4,3}$=5.4						92*
ICS	−0.2	−1.4	−1.4						
22 (HC)	5.09 s $J_{2,3}$=0.9	4.00 m $J_{3,4}$=2.5	α4.84 d $J_{4,3}$=2.5	6.12 d $J_{6,8}$=2.5	6.20 d $J_{8,6}$=2.5				82*
ICS	+0.2	−0.2	+2.0						
23 (HC)	5.47 s	5.16 dd	β4.48 d $J_{4,3}$=2.4	6.60 d $J_{6,8}$=2.4	6.77 d $J_{8,6}$=2.4	7.23	7.33	7.33	92*
ICS	+0.5	−0.2	+2.7						
24 (HC)	5.48 s	5.14 dd	α4.48 d $J_{4,3}$=2.4	6.64 d $J_{6,8}$=2.0	6.68 d $J_{8,6}$=2.0				75*
ICS	+0.5	−0.2	+2.7						
25 (HC)	5.28 s	3.96 m	α4.08 d $J_{4,3}$=2	5.90 d $J_{6,8}$=2	6.04 d $J_{8,6}$=2	7.03 $J_{2',5'}$=2	6.83 $J_{5',2'}$=2	6.4 $J_{6',2'}$=2 $J_{6',5'}$=8	91**
ICS	+0.3	−0.2	+1.3						

*Solvent $CDCl_3$; phenol groups methylated; the underlined figures mean that the hydroxy group was acetylated.

**Solvent acetone-d_6.



21.R= α OH
22.R= β OH
23.R= α Phl
24.R= β Phl
25.R= β S-Ph

Fig. 8. Structures of (−)-epicatechin derivatives having a substituent in position 4.

The additional acetylation of the C-3-OH group causes the following changes in the CSs of the protons of ring *C* (ppm): H-2 (+0.6), H-3 (+1.3), H-4α (−0.1), and H-4β (+0.2). The spectrum of (**9**) was obtained at 100°C [73], but, according to estimates of SSCCs, under these conditions ring *C* retains a conformation close to C2-pentacoplanar. The negative sign of the ICS for the 4α-proton, which has a pseudoequatorial orientation, may be caused by the influence of the spatially anisotropic acetyl group at C-3, which is also oriented equatorially.

Derivatives galloylated in the C-3 position are frequently found among natural flavan-3-ols and proanthocyanidins. Details of the PMR spectra of (+)-catechin and (−)-epicatechin 3-O-gallates (**13** and **14**, respectively) are given in Table 3. A comparison of the CSs of galloylated (+)-catechin [76] and of the initial (+)-catechin has shown that in this case the ICSs are as follows (ppm): H-3 (+1.4), H-2 (+0.6), H-4α (−0.1), and H-4β (+0.2). They are close to the shifts induced by an acetyl group in cyclic alcohols [77] and practically coincide with the acetylation effects discussed above. That is, the effects of the acylation of an OH group in cyclic six-membered alcohols are universal and depend little on the type of acid residue. They can be used in the interpretation of the PMR spectra of this fairly widely distributed group of compounds. A study of the literature on SSCCs has shown that the effects observed are not linked with appreciable conformational isomerism in ring *C*. These phenomena, including the negative value of the ICS for H-4α, are obviously due to an influence on chemical shifts of the redistribution of the electron density in the molecule and also to the influence of the spatially anisotropic carbonyl group of the ester bond.

Galloylation of the phenolic hydroxy group at C-7 of (+)-catechin [68] induces paramagnetic shifts of the protons of ring *A* in the *ortho*- position: H-6 (+0.4 ppm) and H-8 (+0.2 ppm). In addition, a diamagnetic shift (−0.2 ppm) is observed

for the H-4α proton, which has a pseudoequatorial orientation. As the SSCCs show, this effect, also, is not connected with conformational isomerism of the heterocycle. Obviously, it is also caused by the influence of the spatially anisotropic carbonyl group of gallic acid.

Thus, the influence of the formation of ether and ester bonds on the parameters of the PMR spectra of flavan-3-ols with the 2,3-*trans*- configuration has been considered.

Influence of the Glycosylation of Hydroxy Groups on the Parameters of the PMR Spectrum of (+)-Catechin

Glycosides of phenolic compounds are fairly widely distributed in Nature. We shall therefore dwell in more detail on the effect of the glycosylation of the hydroxy groups of flavan-3-ols. According to results published in the literature on the SSCCs of the protons [106], on the glycosylation of the C-3-OH group ring *C* of (+)-catechin assumes a half-boat conformation. And although in this conformation a voluminous substituent at C-3 is oriented axially, it is at the greatest possible distance from the substituent at C-2, which also has a large effective volume. The maximum separation of the substituents under consideration is also apparently responsible for the existence of this, at first sight, unstable conformation (Fig. 5).

The glycosylation effects calculated from results given in [78] amount to +0.4 ppm for H-2 and −0.2 ppm for H-4α. Both protons are present in α-positions with respect to the hydroxy group undergoing glycosylation. Nevertheless, the glycosylation effects observed for them have opposite signs, which is connected with their opposite orientations in relation to the glycosylated group.

A comparison of literature results for the PMR spectra of the 3′-, 4′-, 5-, and 7-glycosides and the 3′,5-, 4′,5-, 3′,7-, and 3′,4′-diglycosides of (+)-catechin [54, 71, 79] has led us to the following conclusions: on the glycosylation of the phenolic OH groups, ring *C* of (+)-catechin retains the C2-pentacoplanar conformation, and the signals of protons present in the *ortho*-positions to the phenol group undergoing glycosylation experience paramagnetic shifts of +0.2 to +0.4 ppm. For the diglycosides, the principle of the additivity of ICSs is observed.

Influence of Substituents in the C-6 and C-8 Positions of (+)-Catechin on the CSs of the Protons of Ring A

The products of the degradation of the proanthocyanidins are often C-6, C-8, and C-4 derivatives of flavan-3-ols. Their investigation permits us to determine the position of the interflavan bond. Some of them have also been isolated from plant materials.

As a rule, the study of 6- and 8-substituted catechins [54, 68, 70, 80-85] follows the aim of distinguishing them from one another. For this, the CSs of the unsubstituted H-6 proton in the C-8 derivatives and H-8 in the C-6 derivatives are used. Figures for corresponding pairs of methyl ether derivatives are given in Table 4.

As follows from Table 4, the CS of an unsubstituted H-8 proton is 0.1-0.3 ppm greater than the CS of H-6 in the corresponding derivatives. Many authors have used this difference to distinguish derivatives in the corresponding pairs. However, this rule is not valid for all types of derivatives, which is apparently connected with their different effects on the chemical shifts of the protons in the ring under consideration. As an analysis of the literature on the investigation of corresponding pairs of (+)-catechin and (−)-epicatechin derivatives [54, 86] has shown, for the phenols themselves this rule is not clearly expressed and the CSs of the corresponding protons must not be used in the identification of 6- and 8- derivatives of the phenols themselves.

Characteristics of the PMR Spectra of 4α- and 4β-Substituted Flavan-3-ols with the 2,3-*trans*- Configuration

Flavan-3-ols having substituents in the C-4 position are products that can be obtained in the degradation of proanthocyanidins (Fig. 6). Their investigation is therefore of great value. Parameters of the PMR spectra of this group of compounds are given in Table 5.

One of the groups of such products consists of the catechin 4α-phloroglucinol derivatives [56, 69, 87-90]. They also fulfil the role of model compounds in the interpretation of PMR spectra of proanthocyanidins.

Analysis of the parameters of the PMR spectra of 4α-phloroglucino-(+)-gallocatechin (**15**) and its peracetate (**15a**) and also of 4β-phloroglucinol derivatives of flavan-3-ols (**16**, **16a**) has shown that the α-, β-, and γ-effects of phloroglucinol in position 4 are, in the first case — the α- isomer — +2.2, 0.3, and 0.0 ppm, respectively, and in the second case — the β- isomer — +2.1, 0.0, and −0.8. At the same time, for a given derivative the chemical shifts of the H-2 and H-4 protons may differ by up to 0.2 ppm. These differences may be due to hindered rotation around the sp^3-sp^2 bond of the spatially anisotropic aromatic rings, since, according to the spin-spin coupling constants there is no isomerism in ring *C*.

It follows from SSCC results [89, 90] that, under normal conditions, ring *C* of the α- derivatives has a close to half-chair conformation, and the β- derivatives a C2-pentacoplanar or half-boat conformation. Such conformations are due to the orientation of the voluminous substituent at C-4, striving to assume the energetically most favorable equatorial orientation (Fig. 7).

The SSCCs of the analogous derivatives of (+)-fisetinidol (3,3′,4′,7-tetrahydroxyflavan) [56, 90] show that, as also in (+)-gallocatechin derivatives, the α- analogs have a half-chair conformation, and the β- derivatives a C2-pentacoplanar or half-boat conformation. In these cases, the substituent at C-4 has a close to equatorial orientation. It follows from the facts discussed above that a substituent at C-4 strives to assume the equatorial conformation, which largely determines the conformation of ring *C*.

A generalization of results reported in the literature of the study of 4-hydroxy derivatives of flavan-3-ols with the 2,3-*trans*- configuration [29, 89, 92-96] has shown that the 4α- analogs have a C2-pentacoplanar configuration, and the β- derivatives a half-chair conformation. The scatter of the ICSs for H-4 of identical derivatives calculated from different literature sources may amount to 0.3 ppm. As the SSCCs show, it is not connected with conformational isomerism of ring *C*.

A study of the literature on the ^{1}H NMR spectra of (+)-gallocatechin [75], (+)-physetinidol [55, 56, 70, 90, 94, 108], and afzelechin [55, 94, 96, 97] has shown that the degree and type of oxidation of the aromatic rings affect the chemical shifts of the protons of ring *C* to some degree, but have no influence on the SSCCs. That is, an influence on chemical shifts is most frequently not connected with conformational isomerism in ring *C*. It is apparently due to the spatial influence of the anisotropic aromatic ring *B*.

Regardless of the orientation of the substituent, compounds with thioether groups in the C-4 position have a conformation close to C2-pentacoplanar.

Thus, we have considered the influence that substituents in position 4 exert on structural and spectral parameters.

Spectra of Flavan-3-ol Derivatives with the 2,3-*cis*- Configuration

Characteristics of the PMR Spectra of Ethers

On the methylation of the phenolic hydroxy groups [69, 109] no appreciable changes in the spectral parameters of ring *C* are observed. Analysis of SSCCs shows that (−)-epicatechin methylated in the aromatic hydroxy groups has a half-chair conformation. The influence of methylation of the phenolic hydroxy groups on the aromatic protons has a fairly complex nature. Thus, methylation of the hydroxy group at C-3′ leads to downfield shifts of the signals of the protons in the *ortho*- position — H-2′ — by +0.15 ppm and in the *para*- position — H-6′ — by +0.1 ppm, and also to a diamagnetic shift of the H-5′ proton, occupying a *meta*- position in relation to the hydroxy group undergoing methylation, by −0.1 ppm. In this case, the observed effects are close to those found on the methylation of the OH group of phenol.

Methylation of the C-5-OH group induces diamagnetic shifts of the H-6 proton by −0.1 ppm and of the H-8 proton by −0.2 ppm; i.e., the methylation of phenol groups leads to paramagnetic shifts of the signals of the *ortho*- and *para*- protons. In this case, the ICS amounts to +0.3 ppm. It also causes a diamagnetic shift of the protons in the *meta*- position by −0.2 ppm.

According to SSCC results [69, 98], acetylation of the C-3-OH group does not affect the conformation of heterocycle *C*. It retains the half-chair form. The influence of acetylation on the CSs of the protons of this ring bears the same nature as in (+)-catechin.

However, the induced diamagnetic shift of H-2 by +0.1 ppm is fairly small in comparison with the corresponding parameter in (+)-catechin, +0.6 ppm. This phenomenon is apparently connected with the absence of a descreening influence of the spatially anisotropic carbonyl group, which, in a 2,3-*cis*- isomer, is remote from the H-2 proton.

Literature information on the PMR spectra of C-3-O-gallates of (−)-epigallocatechin [50, 51, 69] shows some scatter of the ICSs, which is apparently due to hindered rotation around the ester bond. However, the order and sign of the effects of galloylation are close to the corresponding magnitudes observed in the 3-O-acetates.

The generalization that we have made of literature information on the PMR spectroscopy of derivatives of (−)-epicatechin, (−)-epigallocatechin, (−)-epifisetinidol, and (−)-epiafzelechin [51, 55, 69, 86, 95, 99, 100] has permitted the conclusion that the degree of oxidation of the aromatic rings has no substantial influence on the spectral parameters of ring *C* either of the phenols themselves or of their ethers and esters. The methylation of phenol groups leads to paramagnetic shifts of the signals of the aromatic protons present in *ortho-* or *para-* positions to the hydroxy groups undergoing methylation.

Influence of Substituents at C-4, C-6, and C-8 on the Parameters of the PMR Spectra of 2,3-*cis*-Flavan-3-ols

4-β- Derivatives of (−)-epicatechin and its stereochemical analogs are encountered fairly frequently. Natural 4-β-phloroglucino-(−)-epicatechin was first isolated by Kolodziej [84], and the 2,3-*cis*-3,4-*cis*- analog by van der Westuzen [101].

In spite of steric hindrance, derivatives of (−)-epicatechin and its stereochemical analogs exist in which a substituent at C-4 has the α- orientation [57, 75, 92, 102, 103] (Fig. 8).

These substances can most frequently be distinguished from their β- analogs by SSCCs (see Table 6), with allowance for possible conformational isomerism and also with the use of proton chemical shifts.

Thus, the SSCC $^3J_{3,4}$ in the α-hydroxy derivative (**21**) is 4.8 Hz, while in the β- diasteromer (**22**) it is 2.5 Hz [92]. In the 4-OH derivatives the signal of the H-2 proton of the 4-β- compound is shifted by 0.2 ppm downfield, and the signals of the H-3 and H-4 protons by 0.2 and 0.3 ppm, respectively, upfield as compared with the signals of the corresponding protons of the 4-α- analog.

The effects on the H-2, H-3, and H-4 CSs of the simultaneous acetylation of the 3- and 4-OH groups in these two types of compounds also differ somewhat: in the 4-α- derivative they amount to +0.2, +1.4, and +1.3 ppm, and in the β- analog to 0.0, +1.2, and +1.2 ppm. (In this case the principle of additivity of the effects of acetylation is observed.) Hence, in the case under consideration, the ICSs for H-2, H-3, and H-4 induced by a substituent in position 4 can also be used for determining the stereochemistry of substituents in ring *C*.

If it is not possible to use SSCC values for determining stereochemistry in ring *C*, one may use the chemical shifts of the protons alone. For example, in the peracetate of the 4α-phloroglucinol derivative (**23**) the CSs of the H-2, H-3, and H-4 protons are, respectively, 5.48, 5.14, and 4.48 ppm [75], and in the 4β- analog (**24**) they are, respectively, 5.28, 4.97, and 4.31 ppm [64], while the values of $J_{3,4}$ in this pair of diastereomers differ by only 0.1 ppm, i.e., they practically coincide.

A comparison of the CSs of the H-6 proton in C-8 derivatives and of the H-8 proton in C-6 derivatives of (−)-epicatechin made by a number of authors has shown that in those cases where the substituent is bromine the H-8 CS is larger than the H-6 CS for the corresponding pairs of derivatives [80, 82, 104, 105]. When the substituent is isoprenyl, C-β-glucose, or iodine [54], and also in the gambiriins [68, 85, 107] the pattern is reversed: the signal of the H-6 proton for the corresponding pairs of derivatives appears in a weaker field than the signal of the H-8 proton. That is, the nature of the influence of substituents in position 6 or 8 of (−)-epicatechin is the same as that observed in (+)-catechin (see above). This means that the stereochemistry of ring *C* of the catechins can affect the nature of the shifts induced by substituents in aromatic ring *A*.

Thus, we have made a practically complete analysis of the PMR spectra of flavan-3-ols and their derivatives, have revealed spectral correlations in connection with their structural factors, and have noted extremely important features of the PMR spectra of the flavan-3-ols. This permits the determination of structures, the establishment of relative configurations, and, in many cases, the ascertainment of the conformations of compounds of this fairly broad class.

REFERENCES

1. K. Markham, Techniques of Flavonoids Identification, Academic Press, New York (1982).
2. G. G. Zapesochnaya, Khim. Prir. Soedin., 1 (1982).
3. T. J. Mabry and K. R. Markham, The Systematic Identification of Flavonoids, Springer, New York (1970).

4. A. Pelter, R. S. Ward, and T. Lan Grey, J. Chem. Soc, Perkin Trans. I, No. 23, 2475 (1986).
5. P. K. Agrawal and P. Rastogi, Heterocycles, **16**, 2183 (1981).
6. J. Emsley, J. Feeney, and L. Sutcliffe, High Resolution NMR Spectroscopy, Pergamon, Oxford, Vol. I (1965), Vol. II (1966).
7. R. Drago, Physical Methods in Chemistry, Reinhold, New York (1965).
8. N. S. Bhacca and D. Williams, Application of PMR in Organic Chemistry, Holden-Day, San Francisco (1964).
9. B. I. Ionin, B. A. Ershov, and A. N. Kol'tsov, NMR Spectroscopy in Organic Chemistry [in Russian], Khimiya (Leningrad division) (1983).
10. V. Filipoborn, Usp. Khim., **43**, 455 (1974).
11. L. M. Jackman and S. Sternhel, Application of NMR Spectroscopy in Organic Chemistry, Pergamon, Oxford (1969).
12. G. A. Morris, Magn. Reson. Chem., **24**, 371 (1986).
13. W. McFarlane, Annu. Rep. Progr. Chem., **B 80**, 3 (1980).
14. E. Breitmaier and W. Voelter, ^{13}C NMR Spectroscopy, Verlag Chemie, Weinheim (1978).
15. I. Fleming and D. H. Williams, Spectroscopic Methods in Organic Chemistry, McGraw-Hill, New York (1966).
16. R. K. Harris, NMR Spectroscopy, 1 (1971).
17. E. Deroum, Modern Methods of NMR for Chemical Investigations [Russian translation], Mir, Moscow (1962).
18. Yu. A. Zhdanov and V. I. Minkin, Correlation Analysis in Organic Chemistry, Rostov (1966), p. 409.
19. J. B. Stothers, Carbon-13 NMR Spectroscopy, Academic Press, New York (1972).
20. G. Le Coeq and J. Lallemand, J. Chem. Soc., Chem. Commun., 150 (1981).
21. E. Haslam, Phytochemistry, **16**, 1625 (1977).
22. E. V. Brandt, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, No. 7, 1879 (1981).
23. J. P. McManus, R. G. Davis, T. H. Lilley, and E. Haslam, J. Chem. Soc., Chem. Commun., No. 7, 309 (1981).
24. T. Goodwin and E. Mercer, An Introduction to Plant Biochemistry, 2nd Ed., Pergamon, Oxford (1982).
25. A. I. Ismailov, A. K. Karimdzhanov, Sh. Yu. Islambekov, and Z. B. Rakhimkhanov, Khim. Prir. Soedin., 19 (1994).
26. V. I. Vakhrushev, The Production of Tanning Extracts [in Russian], Moscow (1990).
27. Sh. Yu. Islambekov, A. K. Karimdzhanov, S. M. Mavlyanov, and A. I. Ismailov, Khim. Prir. Soedin., 293 (1990).
28. E. C. Bate-Smith, Phytochemistry, **19**, 985 (1980).
29. H. A. Stafford, H. H. Lester, and J. Porter, Phytochemistry, **24**, 333 (1985).
30. J. McManus, K. G. Davis, J. E. Beart, S. H. Gabfnay, and T. H. Lilley, J. Chem. Soc., Perkin Trans. I, No. 9, 1429 (1985).
31. L. Y. Foo, J. Chem. Soc., Chem. Commun., 675 (1986).
32. Z. Czohanska and L. Y. Foo, Phytochemistry, **19**, 1815 (1980).
33. E. C. Bate-Smith, Phytochemistry, **12**, 907 (1973).
34. M. Emsemeyer, L. Lanhammer, and H. Rauwald, Arch. Pharm., **313**, 61 (1980).
35. P. E. Laks, Phytochemistry, **26**, 1617 (1987).
36. M. Takechi, Y. Tanaka, M. Takehara, G. Nonaka, and I. Nishioka, Phytochemistry, **24**, 2245 (1985).
37. N. Kakinchi, M. Hattori, T. Namba, M. Nishizawa, T. Yamagishi, and T. Okuda, J. Nat. Prod., **48**, 614 (1985).
38. W. Herrick, J. Agric. Food. Chem., **28**, 228 (1980).
39. J. Harborne, An Introduction to Ecological Biochemistry, Academic Press, London (1977).
40. S. A. Ostroumov, Introduction to Biochemical Ecology [in Russian], izd. MGU, Moscow (1986).
41. M. J. Brandon, L. Y. Foo, L. J. Porter, and P. Meredith, Phytochemistry, **21**, 2953 (1982).
42. H. A. Stafford, Phytochemistry, **22**, 2643 (1983).
43. L. F. Tilstra, H. Maeda, and W. L. Mattice, J. Chem. Soc., Perkin Trans. I, 1613 (1988).
44. T. Kasuge and H. Ishida, Chem. Pharm. Bull., **33**, 1503 (1985).
45. Yu. A. Ovchinnikov, Bioorganic Chemistry [in Russian], Moscow (1988).
46. A. U. Berg, D. P. Baron, and P. A. Berg, Int. J. Immunopharmacol., **10**, 367 (1988).
47. Z. Czohanska, L. Y. Foo, R. H. Newman, and L. J. Porter, J. Chem. Soc., Perkin Trans. I, 2278 (1980).
48. R. S. Thompson, D. Jacques, E. Haslam, and R. J. N. Tanner, J. Chem. Soc., Perkin Trans. I, 1387 (1972).
49. G. Nonaka and I. Nishioka, Chem. Pharm. Bull., **30**, 4268 (1982).
50. G. Nonaka, R. Sakai, and I. Nishioka, Phytochemistry, **23**, 1753 (1984).
51. G. Nonaka, O. Kawahara, and I. Nishioka, Chem. Pharm. Bull., **31**, 3906 (1983).

52. S. Morimoto, G. Nonaka, I. Nishioka, N. Ezaki, and N. Takizawa, Chem. Pharm. Bull., **33**, 2281 (1985).
53. A. C. Huitric, J. B. Carr, W. F. Trager, and B. J. Nist, Tetrahedron, **19**, 2145 (1963).
54. Y. Kashiwada, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **34**, 3208 (1986).
55. J. W. Clark-Lewis, Austr. J. Chem., **21**, 2059 (1968).
56. S. E. Drewes, D. J. Roux, J. Feeney, and H. Eggers, J. Chem. Soc. (C), 1217 (1967).
57. N. Tanaka, T. Murakami, H. Wada, A. B. Gutierres, Y. Saiki, and C. Chen, Chem. Pharm. Bull., **33**, 5231 (1985).
58. K. Weinges, W. Bahr, W. Ebbert, K. Göritz, and H. D. Marks, Forsch. Chem. Org. Naturst., **27**, 158 (1969).
59. S. E. Drewes, D. J. Roux, J. Feeney, and H. Eggers, J. Chem. Soc., Chem. Commun., 368 (1966).
60. B. J. Bolger, A. Hirwe, K. R. Marathe, E. M. Philbin, M. A. Vickars, and Lillia, Tetrahedron, **22**, 621 (1966).
61. A. J. Birch, C. J. Dahl, and A. Pelter, Tetrahedron Lett., 481 (1967).
62. D. Ferreira H. K. L. Hundt, and D. J. Roux, J. Chem. Soc., Chem. Commun., 1257 (1971).
63. K. Weinges, K. Göritz, and F. Nader, Liebigs Ann. Chem., **715**, 164 (1968).
64. A. Steencamp, J. C. S. Malan, and D. Ferreira, J. Chem. Soc., Perkin Trans. I, 2179 (1988).
65. K. Blaga, O. Chervinka, and Ya. Kovar, Principles of Stereochemistry and Conformational Analysis [in Russian], Khimiya, Moscow (1994), p. 78.
66. H. Kolodziej, Phytochemistry, **25**, No. 9, 1209 (1986).
67. V. Dashevskii, Conformations of Organic Molecules [in Russian], Khimiya, Moscow (1974), p. 188.
68. N. Tanaka, G. Nonaka, and I. Nishioka, Phytochemistry, **22**, 2575 (1983).
69. J. W. Clark-Lewis, L. M. Jackman, and T. M. Sortswood, Austr. J. Chem., **17**, 632 (1964).
70. J. J. Bota, P. M. Viviers, D. Ferreira, and D. J. Roux, Phytochemistry, **21**, 1289 (1982).
71. M. Takani, M. Nakano, and K. Takahashi, Chem. Pharm. Bull., **25**, No, 12, 3388 (1981).
72. G. Delgado, L. Alvares, and A. R. de Vivar, Phytochemistry, **23**, 675 (1984).
73. A. Delcour, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 1711 (1983).
74. M. Miyamura, G. Nonaka, T. Tomimatsu, and I. Nishioka, Phytochemistry, **22**, 215 (1983).
75. L. Y. Foo and L. J. Porter, J. Chem. Soc., Perkin Trans. I, 1186 (1978).
76. E. Haslam, J. Chem. Soc. (C), 1824 (1966).
77. S. Morimoto, G. Nonaka, R. Chen, and I. Nishioka, Chem. Pharm. Bull., **36**, 39 (1988).
78. B. Zhang, G. Nonaka, and I. Nishioka, Phytochemistry, **27**, 3277 (1988).
79. L. Lanhammer, G. Schulze, R. Gujer, D. Magnolato, and M. Horisberger, Planta Med., **49**, 181 (1983).
80. H. K. L. Hundt and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 1727 (1981).
81. F. Bohlman, J. Jacupovic, R. M. King, and H. Robinson, Phytochemistry, **19**, 181 (1980).
82. H. Kolodziej, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 343 (1984).
83. G. W. McGraw and R. W. Hemingway, J. Chem. Soc., Perkin Trans. I, 973 (1984).
84. H. Kolodziej, Tetrahedron Lett., **24**, 1825 (1983).
85. G. Nonaka and I. Nishioka, Chem. Pharm. Bull., **28**, 314 (1980).
86. S. Morimoto, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **34**, 633 (1986).
87. J. J. Bota, D. Ferreira, and D. J. Roux, J. Chem. Soc., Chem. Commun., 698 (1978).
88. S. E. Drewes, D. J. Roux, H. M. Saayman, H. Eggers, and J. Feeney, J. Chem. Soc (C), 1302 (1967).
89. L. J. Porter and L. Y. Foo, Phytochemistry, **21**, 2947 (1982).
90. J. J. Bota, D. Ferreira, and D. J. Roux, J. Chem. Soc., Chem. Commun., 700 (1978).
91. F. Hsu, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **33**, 3293 (1985).
92. M. I. Baig, J. W. Clark-Lewis, and M. J. Thompson, Austr. J. Chem., **22**, 264 (1969).
93. M. I. Baig, J. W. Clark-Lewis, R. W. Jemison, and M. J. Thompson, J. Chem. Soc., Chem. Commun., **D14**, 820 (1969).
94. I. C. du Prekez, D. Ferreira, and D. J. Roux, J. Chem. Soc., (C), 236 (1971).
95. J. W. Clark-Lewis and L. R. Williams, Austr. J. Chem., **20**, 2151 (1967).
96. F. Bohlman, C. Zdero, R. M. King, and H. Robinson, Phytochemistry, **18**, 1246 (1979).
97. F. Hsu, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **33**, 3142 (1985).
98. W. Mayer, L. Goll, E. M. van Arndt, and A. Mannschreck, Tetrahedron Lett., 422 (1966).
99. G. Nonaka, N. Miwa, and I. Nishioka, Phytochemistry, **21**, 429 (1982).

100. L. Y. Foo, L. Hrstich, and C. Vildain, Phytochemistry, **24**, 1495 (1985).
101. J. H. van der Westuzen, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 1220 (1981).
102. J. W. Clark-Lewis, T. McL. Spotswood, and L. R. Williams, Austr. J. Chem., **16**, 107 (1963).
103. J. W. Clark-Lewis and M. M. Mahandru, Austr. J. Chem., **24**, 549 (1971).
104. H. K. L. Hundt and D. J. Roux, J. Chem. Soc., Chem. Commun., 696 (1978).
105. P. H. Viviers, H. Kolodziej, D. A. Young, D. Ferreira, and D. J. Roux, J. Chem. Soc., Perkin Trans. I, 2555 (1983).
106. E. Kiehlman, N. Lehto, and D. Cherniwchan, Can. J. Chem., **66**, 2431 (1988).
107. J. P. Steynberg, D. Ferreira, and D. J. Roux, Tetrahedron Lett., **24**, 4147 (1983).
108. L. Y. Foo and J. Porter, J. Chem. Soc., Perkin Trans. I, No. 7, 1535 (1983).
109. M. Miyamura, G. Nonaka, T. Tomimatsu, and I. Nishioka, Phytochemistry, **22**, 215 (1983).
110. Sh. Yu. Islambekov, A. K. Karimdzhanov, A. I. Ismailov, F. G. Kamaev, and A. S. Sadykov, Khim. Prir. Soedin., 46 (1976).
111. Fam Fam Tkhan', B. Maksudova, and O. S. Otroshenko, Khim. Prir. Soedin., 394 (1981).
112. J. B. Harborne and T. J. Mabry, The Flavonoids, Advances in Research, Chapman and Hall, London (1982).